USE OF N-PIPERIDINE DERIVATIVES FOR THE TREATMENT OF NEURODEGENERATIVE
PATHOLOGIES

It is known that oxygen-centred free radicals (ROS) and
nitrogen-centred free radicals (RNS) are regularly generated
during cell respiration and normal metabolism, performing
important physiological functions such as, for example, that
of second messengers in the induction of cell processes
(Suzuki H.J. Free Rad. Biol. Med. *22, 269-285, 1977*; Clement
M.V. & Pervaiz S. Free Rad. Res. 30, 247-2525, 1999).

It is also known that a large and increasing number of
pathological processes are associated with an increase in the
generation of reactive radical species such as the superoxide
anion ($O_2^{•-}$), the perhydroxyl radical ($HO_2^{•-}$), the hydroxyl
radical ($HO^{•}$), the oxygen singleton, ($^{1\Delta}O_2$), hydrogen peroxide
(H_2O_2), nitric oxide ($NO^{•}$), nitrogen dioxide (NO_2^{-}),
peroxynitrite and other ($R^{•}$) radicals (alkyl-$L^{•}$, alkoxy-$LO^{•}$,
peroxy-$LOO^{•}$ radicals, etc.).

The radicals which are produced in excess, saturating the
antioxidizing machinery that is constituted both by enzymatic
systems (superoxide dismutase, glutathione peroxidase,
catalase, glutathione reductase) and by hydrophilic and
lipophilic non-enzymatic systems (ubiquinone, ubiquinol,
vitamin E, vitamin C, vitamin A, reduced glutathione, uric
acid, carotenoids etc.) generate the much-proclaimed
"oxidative stress state" (OSS) which is responsible for cell
death and tissue damage: radical attack can result in a
further increase in ROS, RNS and $R^{•}$, that is, in OSS, with
consequent exacerbation of the initial oxidative damage (Diaz
M.D. *et al.,* New Engl. J. Med. 331, 408-416, 1997; Cerutti
P.A., The Lancet 344, 862-863, 1994; Giacosa A. & Filiberti
R. Eur. J. Cancer Prev. 5, 307-312, 1996; Bruce N.A. *et al.*

Proc. Natl. Acad. Sci. (USA) 90, 1915-1922, 1993; Albens L.
et al. J. Neural Transm. (Suppl.) 59, 133-154, 2000; Foley M.
et al. J. Neurol. (Suppl. 2) 247, II82-94, 2000; Huang Z. et
al. Can J. Neurol. Sci. (Suppl. 1) 30, S10-18, 2003; He Y. et
al. J. Neurochem. 86, 1338-1345, 2003; Gao H.M. et al. Trends
Pharmacol. Sci. 24, 395-401, 2003; Sekhon B. et al. Brain
Res. 971, 1-8, 2003; Nedelykovic Z.S. et al. Postgrad. Med.
J. 79, 195-198, 2003; Arbiser J.L. Nat. Med. 9, 1103-1104,
2003; see also Medline® and Toxline®).

Furthermore, it is known to try to attenuate the cascade of
events which leads to OSS by various therapeutic approaches
each with inevitable problems which preclude or limit its use
in man or in animals. Chelating agents which, for example,
can catalyze undesired redox reactions in the cells, easily
dissociate or lose activity as a result of bonds with
multiple cell components (Ikeda Y. et al. Neurosurgery 24,
820-824, 1989; White B.C. & Krause G.S. Ann. Emerg. Med. 22,
970-979, 1993).

Apart from the limiting factor connected with enzyme
saturation (≤ turnover number) and with the individual
dismutate reactive species ($O_2^{\bullet-}$) in the more complex and
dynamic world of OSS, the use of the enzyme superoxide
dismutase poses problems of stability (even when included in
liposomes) with a half-life variable between a few minutes
and several hours according to type; it does not pass through
the cell membranes and even less through the blood-brain
barrier, and is immunogenic (although the recent use of
recombinant human r-h-MnSOD forms seems encouraging) (Mikawa
S. et al. J. Neurosurg. 85, 885-891, 1996; Kontos H.A. & Wei
E.P. J. Neurosurg. 64, 803-807, 1986; Chan P.H. et al. Ann.
Neur. 21, 540-547, 1987); it is not without significance
that a recent randomized and multi-centre trial which

provided for the use of glycol-conjugated superoxide dismutase (Pegorgotein®) in closed cranial traumas inevitably did not give the results that were hoped for (Young B. *et al.* JAMA 276, 538-543, 1996).

Some of the limitations connected with the use of the natural enzyme such as, for example, cell permeability - with particular reference to the blood-brain barrier - and administration, have been overcome with the use of organic compounds (for example M40401® or M40403®) with low molecular weight and with catalytic-dismutase activity, for example, towards the superoxide (SOD mimetics) (see US patents Nos. 5 874 421, 5 637 578 and 5 696 109; International applications WO02/28390 and WO02/058686, Samilowski W.E. *et al.* Nat. Med. 9, 750-755, 2003), or even with the use of the isolated active portion of the enzyme (US patent No. 6 117 454).

However, even these strategies can have only limited effectiveness since they reproduce the activity of the natural enzyme at molecular level; in fact they also act on a single radical species ($O_2^{\bullet-}$) and are therefore completely inactive with respect to the others, ROS, RNS and $R^{\bullet}$ which lead to OSS as a whole. At the moment, drugs which need to pass through the blood-brain membrane and which pass through it with difficulty or are unable to pass through it, have to be administered by direct infusion into the CNS or by controlled-release polymer implant, with problems which can easily be imagined (see, for example, US patent No. 4 883 666).

Further molecules which have been proposed are the so-called "spin-trapping scavenging agents" such as, for example, azulenyl nitrones, NXY-059 and, in particular, alpha-phenyl-tert-butyl nitrone (PBN) which has given satisfactory results in various pathologies (Rachnilewitz D. *et al.* Gut 35, 1181-

1188, 1994; Krishna M.C. *et al.* J. Biol. Chem. 271, 26018-26025, 1996; Gilgun-Sherki Y. *et al.* Pharmacol. Rev. 54, 271-284, 2002). Once again, however, the action is limited to a few radical species since, although the action on the carbon-centred radicals ($C^•$) leads to a relatively stable adduct, the bond with oxygen-centred radicals is completely unsatisfactory; suffice it to consider, for example that the half-life of the adduct between the undesirable hydroxyl radical ($HO^•$) and PBN is just 40 seconds (Jansen F.G. *et al.* Free Rad. Biol. Med. 12, 169-173, 1992).

After fragmentation, these non-persistent nitroxides also give rise to further radical species which can propagate or initiate the auto-oxidation reaction. Moreover, PBN does not bind nitrogen-centred radicals (RNS) (Hensley K. *et al.* Int. Rev. Neurobiol. 40, 299-317, 1997). Spin traps which have greater stability *in vitro* (Tempo. Tempol), which can bind superoxide radicals ($O_2^{•-}$) and hydroxyl radicals ($HO^•$), which can self-regenerate (that is, which do not have a suicide action like normal antioxidants), and which can simultaneously block Fenton's reaction, that is, which can terminate radical reactions, have been produced (Samuni *et al.* Biochemistry 30, 555-561, 1991; Samuni *et al.* J. Clin. Invest. 87, 1562-1530, 1991; Li H. *et al.* Free Rad. Biol. Med. 32 712-719, 2002; Krishna M.C. *et al.* J. Biol. Chem. 271, 26026-26031, 1996). However, the protective action of these molecules *in vivo* is limited both by their toxicity and by their half-life which is less than 3 minutes (Laight D. *et al.* Br. J. Pharmacol. 124, 238-244, 1988).

To overcome these difficulties, these spin-traps are in fact used *in vivo* in association with a polynitroxylated macromolecule (polynitroxyl human serum albumin; PNA) which reduces their toxicity, permits "recycling" and, if the dose

used is kept low, prevents vasodilating effects (Kupposomi P.
et al. Biochemistry 35 7051-7057, 1996; Kupposomi P. et
al., Magn. Res. Med. 40, 806-811, 1998); naturally, given
their considerable dimensions, these macromolecules function
only in the extracellular spaces.

Pharmaceutical compositions comprising cyclic hydroxylamines
derived from N-piperidine for the treatment of diseases
connected with an excess production of free radicals have
recently been described in United States patent No. 5 981
548.

A first aspect of the present invention is based on the
recognition of the fact that the cyclic hydroxylamines
derived from N-piperidine which are described in the above-
mentioned US patent No. 5 981 548 can pass through the blood-
brain barrier and can therefore be used in the therapeutic
treatment and prophylaxis of neurodegenerative diseases.

Within the scope of the invention, further compounds have
been identified which are derived from N-piperidine and have
pharmaceutical activity in the treatment of various
pathologies indicated below, and for which no use in the
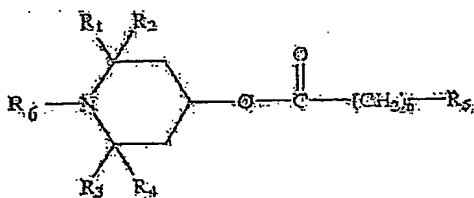pharmaceutical field has been proposed up to now.

With reference to these further compounds, the present
invention provides for novel uses in therapy and prophylaxis
which result from the identification of unexpected properties
thereof such as, in particular, a high capacity to react with
the various types of free radicals, terminating the radical
reactions, the ability to pass easily through the double
lipoprotein layer of the cell membranes (with particular
reference to the blood-brain barrier), a high capacity for
distribution within the tissues, thus enabling a high
concentration of the active substance to be obtained in the

region of the body in which antioxidant protection is required, and the ability to self-regenerate (self-replenishing antioxidants) and to prevent Fenton's reaction by the oxidation of metal ions.

A further object of the present invention is preferably that of providing for novel therapeutic uses with respect to a class of compounds which, in addition to all of the advantages mentioned above, are non-toxic, non-immunogenic, stable and easy to prepare in large quantities at low cost.

The novel therapeutic uses and the pharmaceutical compositions of the invention are defined in the appended claims.
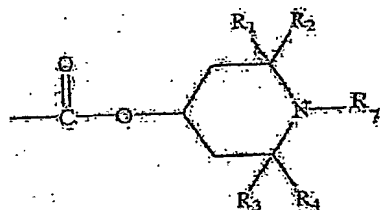
In particular, the novel uses in the pharmaceutical field, which are described in greater detail below, relate to a class of compounds of formula (I):

in which R_6 is oxyl, hydrogen or hydroxyl, R_1, R_2, R_3 and R_4 are selected independently of one another from:
- hydrogen
- alkyl with from 1 to 12 carbon atoms, preferably from 1 to 6 carbon atoms and more preferably from 1 to 3 carbon atoms,
- alkenyl with from 2 to 12 carbon atoms, preferably from 2 to 6 carbon atoms and more preferably from 2 to 3 carbon atoms,

- alkynyl with from 2 to 12 carbon atoms, preferably from 2 to 6 carbon atoms and more preferably from 2 to 3 carbon atoms, or

- R_1 and R_2 together form a tetramethylene or pentamethylene group;

- R_5 is hydrogen,

- alkyl with from 1 to 12 carbon atoms, preferably from 1 to 6 carbon atoms and more preferably from 1 to 3 carbon atoms,

- cycloalkyl with from 3 to 8 carbon atoms,

- alkenyl with from 2 to 12 carbon atoms, preferably from 2 to 6 carbon atoms and more preferably from 2 to 3 carbon atoms,

- alkynyl with from 2 to 12 carbon atoms, preferably from 2 to 6 carbon atoms and more preferably from 2 to 3 carbon atoms, or
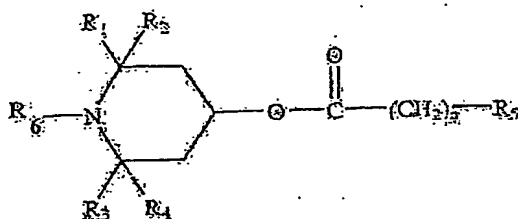

(II)

in which

R_1, R_2, R_3 and R_4 are as defined above, n is a whole number from 1 to 30, more preferably from 2 to 14 and even more preferably from 6 to 10,
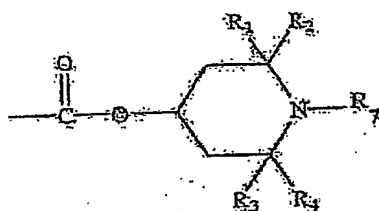
R_7 is hydrogen, oxyl, or hydroxyl.

A group of compounds which are preferred and are illustrative of the present invention are compounds of formula:

in which

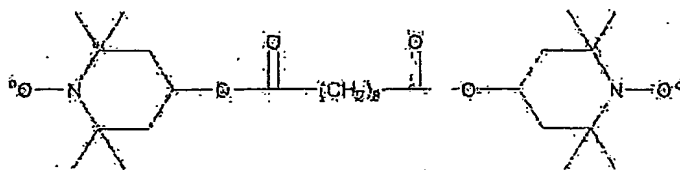R_1, R_2, R_3 and R_4 are alkyl having from 1 to 3 carbon atoms,

R_5 is

(II)

in which

R_1, R_2, R_3 and R_4 are alkyl having from 1 to 3 carbon atoms,

n is a whole number from 1 to 10 and at least one of R_6 and R_7 is oxyl and the other of R_6 and R_7 is oxyl or hydroxyl; in particular, the use of a compound of formula:

known as: bis(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) decandioate is particularly preferred.

The term "alkyl with from 1 to 12 carbon atoms" denotes a substituent group derived from a saturated hydrocarbon by removal of a single hydrogen atom. The term includes methyl, ethyl, n-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl and the various isomeric forms of pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl.

The terms "alkenyl having from 2 to 12 carbon atoms" and "alkenyl with from 2 to 12 carbon atoms" indicate substituent groups derived, respectively, from alkene and alkyne hydrocarbons by removal of a single hydrogen atom. These terms include ethenyl, ethynyl, propenyl, propynyl and similar branched and non-branched unsaturated hydrocarbon groups having up to 12 carbon atoms.

The term "cycloalkyl having from 3 to 8 carbon atoms" indicates saturated carbocyclic rings such as cyclopropyl, cyclobutyl, cyclopentenyl, cyclohexyl, as well as carbocyclic rings substituted with alkyl which contain up to 8 carbon atoms such as, for example, methyl-, dimethyl- and ethylcyclohexyl.

The N-oxyl and N-hydroxyl derivative compounds can be prepared by processes described in US patents Nos. 4 691 015 and 5 981 548, starting with the N-H derivatives.

In particular, the N-oxyl derivatives are obtained from the corresponding N-H derivative by reaction with m-chloroperbenzoic acid. The N-oxyl derivative can be converted into the corresponding N-hydroxy derivative by catalytic hydrogenation, for example, with the use of PtO_2 as catalyst.

As indicated above, a subject of the invention is the use of the above-mentioned compounds for the preparation of medicaments, pharmaceutical or veterinary compositions for the treatment (inhibition, prevention, prophylaxis and therapy) of neurodegenerative syndromes and pathologies such as Parkinson's disease, Alzheimer's disease, encephalic lesions due to ictus, traumatic brain lesions, neuropathy due to HIV, Down's syndrome, diabetic polyneuropathy, muscular dystrophy, multiple sclerosis, Huntington's disease, prion disease, late dyskinesia, tauopathy, demyelinating pathologies and Lou Gherig's syndrome.

The invention also relates to the use of the compounds mentioned for the preparation of pharmaceutical or veterinary compositions and of medicaments for the therapeutic treatment, prevention, and/or prophylaxis of mortal conditions such as cardiac/renal/pulmonary/hepatic/ intestinal ischaemia-reperfusion, hypertension, diabetes, cancer and also of shock, cystic fibrosis, virus infections, toxicity due to drugs and radiation (for example, radiotherapy or radiation protection more generally) inflammation, atherosclerosis, aging, rheumatoid arthritis, epilepsy, hypercholesterolaemia, hyperlipidaemia, as well as in the treatment of pain and sepsis, and pathologies associated with an excess production of free radicals.

For use in the pharmaceutical or veterinary field, the compounds according to the present invention are administered to the patient at dose levels within the range of from 0.01 to 1 g/kg of body weight, preferably from 0.1 to 200 mg/kg and more preferably within a range of between 0.5 and 20 mg/kg of body weight, in one or more daily administrations.

The specific dosages used, however, may vary in dependence on the needs of the patient or of the animal, on the severity of

the pathologies to be treated (on age, sex, diet, method of administration, and on pharmacological considerations such as the activity of the compound to be used, the efficacy and the pharmacokinetic and toxicological profile of the preselected compound and of any combination with other drugs, etc.). The determination of the optimal dose is amongst the possible selections that are open to a person skilled in the art.

Pharmaceutically acceptable vehicles in both solid and liquid form may be used for the preparation of pharmaceutical or veterinary compositions comprising at least one of the compounds according to the present invention.

The methods of administration comprise, in addition to oral and parenteral administration, administration by inhalatory and dermatological (topical) routes.

The solid preparations will include, for example, powders, tablets, granules, capsules, cachets and suppositories.

A solid vehicle may be constituted by one or more substances which may also act as diluents, flavourings, solubilizers, lubricants, suspension agents, binders or disaggregants for tablets; encapsulated material may also be used.

In the powders, the vehicle is constituted by a finely divided solid which is mixed with at least one active compound. In tablets, the active ingredient is mixed with the vehicle having the necessary binding properties in suitable proportions and is compacted in the desired shape and size. The powders and tablets preferably contain between 7 and 70% by weight of the active ingredient.

Suitable vehicles are represented principally by magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin,

dextrin, maize starch, methyl cellulose, sodium carboxymethyl cellulose, waxes with low melting points, coconut butter and the like.

Tablets, powders, cachets and capsules may be used as suitable forms of dosage for oral administration.

Preparations in liquid form include solutions suitable for parenteral administration (subcutaneous, intravenous, intramuscular, intrasternal injections or infusion techniques) or for oral administration, that is, suspensions and emulsions suitable for oral administration. Both sterile aqueous solutions of the active ingredient and sterile solutions of the active ingredient in solvents comprising water, ethanol, or propylene glycol may be mentioned as examples of liquid preparations suitable for parenteral administration.

Sterile solutions may be prepared by dissolving the active ingredient in the desired solvent system and then passing the resulting solution through a membrane filter in order to sterilize it or, alternatively, by dissolving the sterile compound in a previously sterilized solvent, in sterile conditions.

Aqueous solutions for oral administration may be prepared by dissolving the active ingredient in water and adding suitable colouring, flavouring, stabilizing, and agglomerating agents in the desired quantity.

Aqueous suspensions for oral use may be prepared by dispersing the finely divided active ingredient in water together with viscous material such as natural or synthetic gums, resins, methyl cellulose, sodium carboxymethyl

cellulose and other suspension agents known in the art for pharmaceutical or veterinary formulations.

Preferably, the pharmaceutical or veterinary preparation is in the form of single-dose units preferably containing from 1 to 300 mg. In this form, the preparation is divided into unitary doses containing suitable quantities of the active ingredient. The single-dose unit may be constituted by a packaged preparation containing tablets, capsules, or powders in phials or ampoules.

The pharmaceutical compositions of the invention are preferably for use in man. However, since they are usable for man, they may also be useable in the veterinary field for pets, exotic animals and farm animals, including mammals, birds, rodents, etc., more preferably, animals such as dogs and cats, as well as cattle, sheep and pigs.

The following examples may enable a person skilled in the art to implement the invention. They will therefore be illustrative of the invention and are included solely as examples and not as a limitation. The use of the invention both in a model of Parkinson's disease, as an example of a neurodegenerative disorder, and in a model of myocardial ischaemia-reperfusion, as an example of a life-threatening disease, is therefore described.

Example 1

Although Parkinson's syndrome (PS) was described for the first time in 1817 by James Parkinson as a "shaking palsy" of unknown origin (there is, however, evidence of parkinsonian syndromes dating back even thousands of years), its aetiology is still quite obscure.
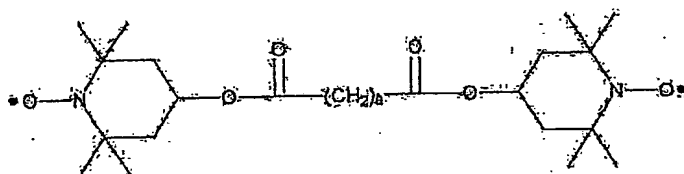
In addition to age, there is lively scientific debate at
international level on the role of genetic and environmental
factors in the origin of PS (Huang Z et al. Can. J. Neur.
Sci. 30 Suppl. 1, 510-518, 2003; Daner W. et al. Proc. Natl.
Acad. Scie. (USA) 99, 14524-14529, 2002; Vaughan J.R. et al
Am. Hum. Gen. 65, 111-126, 2001; Thiruchelvam M. et al.
Neurotoxicology 23, 621-633, 2002; Scott W.K. et al. JAMA
286, 2239-2244, 2001; Warner T.T. & Schapira A.H. Am. Neurol.
53 Suppl. 3, 516-523, 2003). A considerable impulse was
given to research in the environmental field in the middle of
the early nineteen-eighties by the discovery that specific
neurotoxins   such   as   n-methyl-4-phenyl-1,2,3,6-
tetrahydropyridine (MTPT) can cause parkinsonism in man and
in animals; since then, many pesticides have been found to
reproduce PS in animal models (Betarbert R. et al. Nat.
Neurosci. 3, 1301-1306, 2000; Kirbi M.L. et al, Toxicol.
Sci. 61, 100-106, 2001; Yumino K. et al. J. Biochem. (Tokyo)
131, 565-570, 2002; Gao et al. J. Neurosci. 23, 6181-6187,
2003). It is not without significance that case-control
meta-analysis of the studies available suggests the existence
of an association between exposure to pesticides and PS
(Priyadarshi A. et al. Neurotoxicology 21, 435-440, 2000;
Priyadarshi A. et al. Environ. Res. 86, 123-127, 2001).

Much experimental evidence demonstrates that the herbicide
Paraquat (which has a chemical structure similar to MPTP) is
involved in the pathogenesis of PS; both microinfusion of
Paraquat into the Substantia Nigra of the animal and systemic
or intraperitoneal treatment lead to selective degeneration
of dopaminergic neurons accompanied by behavioural and
neuropathological signs of severe non-selective neurotoxicity
(Brooks A.I. et al. Brain Res. 823, 1-10, 1999; Thiruchelvam
M. et al. Neurotoxicology 23, 621-633, 2002; McCornack A.L.
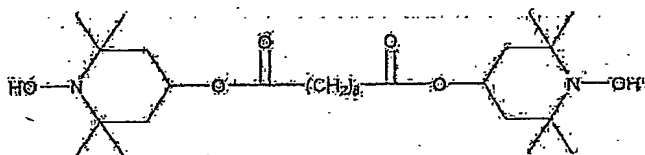et al. Neurobiol Dis. 10, 119-127, 2002) attributed to an

excess of OSS-reactive free radicals (Jannone N. *et al.*
Neuropharmacology 30, 893-898, 1991; Klivenyi G. *et al*
Neurobiol. Dis. 5, 253-258, 1998; Foley M. *et al.* J. Neurol.
247 Suppl. 2, II82-94, 2000).

The Paraquat/rat/PS experimental model was therefore selected
as the most indicative for checking the efficacy of chemical
compounds according to the present invention; of these, two
anti-oxidants designated MP1002 and MP1001 were used in the
tests:

MP1002

MP1001

Male Sprague Dawley rats (210-220 g weight) were kept on a
standard laboratory diet supplemented with "Nossan Pellets"
(both the animals and the pellets are available from Nossan
Company, Milan, Italy) and were housed for three weeks
(relative humidity 50% ± 10%, temperature 22°C ± 1 and in a
light/darkness cycle of 12 hours/12 hours, with first light
at 7.30 a.m.) prior to the tests; food and water were
available *ad libitum*.

Prior to treatment, the rats were anaesthetized by
intraperitoneal injection of 380 mg/kg weight of chloral

hydrate; a stainless steel guide cannula (size 25) was implanted unilaterally in the *Substantia Nigra* with stereotaxic guiding and was secured to the cranium with dental acrylic material.

The animals (20 per test group) were allowed a recovery period of seven days prior to treatment; during this period no change in motor activity or posture was observed. Microinfusions were performed by means of a 10 µl Hamilton syringe connected to an injection cannula by means of a Teflon tube. The compounds or the vehicle (0.8% NaCl) were injected at a total volume of 1 µl/minute.

Microinfusion of 50 µg of Paraquat into the *Substantia Nigra* (n = 20 rats) led to an increase in motor activity with the animals performing jumps and moving in circles in a contralateral direction relative to the injection position, about 24 minutes later; all of the animals were dead 24 ± 2.5 hours after infusion. A further twenty animals were infused with Paraquat and twenty with Paraquat plus vehicle; about ten of the animals for each group survived 20 hours after treatment and were used to determine the development of lipid peroxidation in the *Substantia Nigra*.

When the Paraquat (50 µg) was microinfused into the *Substantia Nigra* simultaneously with 1 µg of MP1002 or MP1001, all of the animals survived for a further 24 hours and no signs of alterations were observed during continuous monitoring (see Table 1). Similar results were obtained after intraperitoneal administration of the anti-oxidants, thus demonstrating the extraordinary capacity of MP1002 or MP1001 to cross the blood-brain barrier (BBB), which is one of the most limiting factors of conventional antioxidants (together with their selectivity in relation to a specific

type   of   radical)   for   therapeutic   purposes   in
neurodegenerative pathologies.

In particular, none of the rats died when: (1) they were pre-
treated intraperitoneally with 120 mg/kg by weight of MP1002
or MP1001 5 minutes prior to microinfusion of 50 µg of
Paraquat;   (2)   they   were   simultaneously   treated   by
intraperitoneal injection of 120 mg/kg by weight of MP1002 or
MP1001 and infused with 50 µg of Paraquat; and (3) they were
microinfused   with   50   µg   of   Paraquat   followed   by
intraperitoneal injection of 120 mg/kg by weight of MP1002
and MP1001 5 minutes later (see Table 1).

Lipid peroxidation in the *Substantia Nigra* was determined by
quantification of the release of malondialdehyde (MDA) which
was   used   as   a   biomarker,   by   means   of   the   reagent
thiobarbituric acid-TCA-HCl, as described by Buege J.A. &
Aust S.D. Meth. Enzymol. 52, 302-310, 1978. Table 1 shows
clearly the increase in lipid peroxidation in the *Substantia
Nigra* in an additional group of animals (about ten) which
survived 20 hours after infusion with 50 µg of Paraquat or
Paraquat and saline solution. Treatment with MP1002 or
MP1001 in the various situations (before, with, or after
infusion with Paraquat) markedly and significantly ($p < 0.01$)
reduced lipid peroxidation in the *Substantia Nigra*, clearly
indicating the high antioxidant potency of those compounds.

In these tests, both the intracerebral administration and the
peripheral administration of MP1002 or MP1001, two types of
antioxidant described herein, protected completely against
the behavioural and neuropathological effects induced by
Paraquat in rats. These results show the capacity of the
compounds according to the invention to cross the brain-blood
barrier easily thus protecting the dopaminergic neurons of

the *Substantia Nigra* against degenerative damage (for example OSS) produced by herbicides such as Paraquat. This represents a useful approach for the treatment of various neurodegenerative pathologies, including Parkinson's disease.

Example 2

The *in vivo* model of myocardial lesion due to ischaemia and reperfusion was selected as the most suitable for checking the efficacy of the compounds MP1002 and MP1001 according to the invention.

Male Sprague Dawley rats (220-230 g) were kept on a standard laboratory diet supplemented with "Nossan Pellets" (Nossan Company, Milan, Italy) and were housed for three weeks prior to the tests (relative humidity 50% ± 10, temperature 22°C ± 1 and light/darkness cycle of 12/12 hours with first light at 7.30 a.m.); food and water were available *ad libitum*.

The rats (twenty per group) were anaesthetized by intraperitoneal injection of chloral hydrate (380 mg/kg) and were then tracheotomized, intubated and ventilated with ambient air by a suitable ventilator; body temperature was kept at 38°C ± 1 and the right carotid artery was cannulated and connected to a pressure transducer to monitor the mean arterial blood pressure (MAP).

The right jugular vein was cannulated for the administration of the compounds, a lateral thoracotomy was performed, the heart was suspended in a temporary pericardial cradle, and a loop occluder was arranged around the left descending coronary artery (LAD); the animals were allowed to stabilize for 40 minutes prior to the LAD ligature.

The coronary artery was occluded by tightening the occluder,
a procedure which is correlated with the electrocardiographic
and haemodynamic changes (drop in MAP) which are typical of
myocardial ischaemia.   After 25 minutes of myocardial
ischaemia, the occluder was reopened, permitting reperfusion
for 2 hours.   The heart rate (HR) and MAP were monitored
continuously.

After reperfusion for 2 hours, the coronary artery was
occluded again and the dye Evans blue was injected (4.5 ml at
2% p/v) into the left ventricle by means of the cannula in
the right carotid artery in order to discriminate between
perfused and non-perfused sections (AAR) of the heart;   the
Evans blue solution dyed the perfused myocardium whereas the
occluded vascular bed remained undyed.

The rats were then killed by an overdose of anaesthetic, the
hearts were removed and were cut into slices (3-4 mm), the
right ventricular wall and the area at risk (pink) was
removed and was separated from the non-ischaemic area (blue).
The area at risk was cut into small pieces and incubated with
tetrazolium p-nitro blue (NBT, 0.48 mg/ml) for 20 minutes at
37°C;   in the presence of intact dehydrogenase enzymes (live
myocardium) the NBT formed a dark blue formazane whereas the
area of necrosis without dehydrogenase activity was not
coloured.   The pieces were separated according to their
coloration and were weighed to determine the size of the
infarct as a percentage of the weight of AAR.

The following test groups were considered: a) LAD occlusion
(25 minutes) and reperfusion (2 hours) plus administration of
the vehicle (saline solution, 3 ml/kg - v - bolus plus 1.8
ml/kg/h) starting 5 minutes after reperfusion and maintained
during the reperfusion period;   b) LAD occlusion and
reperfusion plus administration of MP1002 or MP1001 (10 mg/kg

injection in bolus) 5 minutes prior to reperfusion, followed
by infusion of 2 mg/kg/h during the reperfusion period; c)
sham operation (no LAD occlusion) and infusion of the
vehicle; d) sham operation and infusion of MP1002 or MP1001
as described above.

The malondialdehyde (MDA) in the heart tissue as final
peroxidation product of the cell membrane lipids was also
determined as described above.

Table 2 gives the various MPA and HR values measured during
the course of the tests. The base haemodynamic results were
similar in the various groups considered.

In the animals receiving sham operations (no LAD occlusion)
the infusion of the vehicle or vehicle with MP1002 or MP1001
had no substantial haemodynamic effects.

In the animals which were subjected to coronary occlusion and
reperfusion, the mean MAP values were reduced slightly during
the test period but there were no changes in HR. Moreover,
the mean MAP and HR values of the rats which were subjected
to I/R (ischaemia/reperfusion) treatment and treated with
MP1002 or MP1001 were not significantly different from the
I/R group treated with the vehicle alone.

The infusion of MP1002 or MP1001 brought about a significant
reduction ($p < 0.01$) in the size of the infarct (80% and 78%,
respectively) when compared with the control (Figure 1). The
sham operation did not result in a significant degree of
infarct. Whereas the I/R treatment led to a marked and
significant increase ($p < 0.01$) in lipid peroxidation in
comparison with the rats that were subjected to sham
operations (plus vehicle or antioxidant), treatment with
MP1002 or MP1001 significantly ($p < 0.01$) reduced the

development of peroxidation to levels similar to those of the animals that were subjected to sham operations (Figure 2), thus providing evidence of the high antioxidant power of these compounds.

These tests show that MP1002 and MP1001 can drastically reduce the size of myocardial infarcts after occlusion and reperfusion of the myocardium. These results demonstrate the capacity of the compounds of the invention to exert a good protective effect against myocardial lesions induced by ischaemia-reperfusion.

This suggests that the compounds of the invention offer a novel therapeutic approach for the treatment of life-threatening diseases including ischaemic heart diseases.

TABLE 1. EFFECTS OF EITHER BIS(1-OXYL-2,2,6,6-TETRAMETHYL-4-PIPERIDINYL)DECANDIOATE (MP1002) OR BIS(1-HYDROXYL-2,2,6,6-TETRAMETHYL-4-PIPERIDINYL)DECANDIOATE (MP1001) ON THE LATENCY, DURATION OF SEIZURES, MORTALITY AND LIPID PEROXIDATION DEVELOPMENT IN RATS RECEIVING PARAQUAT INTO SUBSTANTIA NIGRA

Treatment	Latency (min)	Duration (min)	Mortality (%)	Lipid Peroxidation (nmol MDA x h⁻¹ x mg prot⁻¹)
Control	0	0	0	3.8±0.76
Paraquat*	25±4	270±15	100 (20/20)	14.6±1.34**
Paraquat + vehicle*	25±6	266±17	100 (20/20)	13.5±1.10**
Paraquat + MP1002[a]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	4.3±0.63**
Paraquat + MP1001[a]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	5.4±0.84**
Paraquat + MP1002[b]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	5.2±0.75**
Paraquat + MP1001[b]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	4.7±0.49**
Paraquat + MP1002[c]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	4.9±0.86**
Paraquat + MP1001[c]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	5.1±0.94**
Paraquat + MP1002[d]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	4.1±0.39**
Paraquat + MP1001[d]	No seizures and epileptic discharges	No seizures and epileptic discharges	0	4.8±0.57**

In Table 1 each value represents the mean ± SD of twenty different tests performed on twenty different rats; a) animals which received, with simultaneous microinfusion into the *Substantia Nigra*, Paraquat (50 µg) and 1 µg of MP1002 or of MP1001; b) animals which were pretreated intraperitoneally with 120 mg/kg by weight of MP1002 or of MP1001 and which, after 5 minutes, received Paraquat (50 µg) by microinfusion; c) animals which received simultaneously 50 µg of Paraquat by microinfusion and 120 mg/kg by weight of MP1002 or of MP1001 by intraperitoneal injection; d) animals which received 50 µg of Paraquat by microinfusion and, 5 minutes later, 100 mg/kg by weight of MP1002 or of MP1001 by intraperitoneal injection.

Infusion of the vehicle into the same site did not produce any effect in the behavioural changes or in the mortality correlated with the Paraquat.

The table also gives the variations of lipid peroxidation in the *Substantia Nigra* as levels of malondialdehyde (MDA) 24 or 20 (*) hours after treatment. See the text for details and test procedures.

**p < 0.01 Paraquat or Paraquat + vehicle against control and Paraquat + vehicle against MP1002 or MP1001-treated groups (Wilcoxon method of classification).

TABLE 2. EFFECTS OF THE ANTIOXIDANTS MP1002 AND MP1001 ON MEAN ARTERIAL BLOOD PRESSURE (MAP) AND HEART RATE (HR) CHANGES IN MYOCARDIAL ISCHAEMIA AND REPERFUSION (I/R) IN THE RAT

Group		Baseline	Occlusion (min)		Reperfusion (min)	
			15	25	15	25
Sham + vehicle	MAP	128±12	126±14	118±10	112±9	105±12
	HR	425±25	429±26	426±21	431±23	429±22
Sham + MP1002	MAP	125±15	123±16	120±11	110±12	105±9
	HR	421±26	420±33	422±25	428±26	426±32
Sham + MP1001	MAP	127±16	125±13	121±11	112±8	107±16
	HR	422±33	422±26	424±48	427±27	427±23
I/R + vehicle	MAP	128±10	121±12	113±10	101±12	96±9
	HR	445±34	440±31	436±27	434±28	436±26
I/R + MP1002	MAP	127±14	121±14	114±10	102±11	97±9
	HR	438±30	437±32	431±24	427±29	429±25
I/R + MP1001	MAP	125±9	118±12	111±13	100±10	95±8
	HR	435±25	438±31	430±28	425±28	427±26

Each value represents the mean ± SD of at least fifteen different experiments.
Animals received MP1002, MP1001 or an equal volume of vehicle.
See text for details and experimental procedures.